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Functional Ecology

DOI:
10.1111/1365-2435.13576

Published: 01/07/2020

Peer reviewed version

Ocean warming increases the nitrogen demand and the uptake of organic nitrogen of the globally distributed seagrass *Zostera marina*

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Running head: Shifts in *Zostera marina* N uptake with temperature

Abstract

1. The impact of global warming on the metabolic state of a species may be examined by either measuring physiological rates across a latitudinal gradient or by assessing short-term responses under experimentally controlled temperature regimes. The combination of the two approaches is seldom used but it provides valuable information on an organism's responses to temperature at broader temporal and spatial scales while allowing the isolation of temperature effects from other environmental variables.

2. Here we used both approaches to assess the warming effects on the total acquisition of dissolved inorganic nitrogen (DIN; nitrate, ammonium) and organic N (DON; amino acids, peptides) by the globally widespread seagrass *Zostera marina*. DIN and DON uptake rates were measured in plants from three sites covering the species latitudinal distribution in Europe (Iceland, United Kingdom and Portugal). The responses of DIN and DON uptake rates of plants from the middle latitude (UK) to a latitudinal range of temperatures (8, 12 and 17 ºC) were also measured. We further examined the microbial uptake of DON along the latitudinal distribution and whether temperature is the main driver of that uptake.

3. Our results showed that warming greatly increased the total N uptake by *Z. marina* and also the relative contribution of DON to total N acquisition. The microbial uptake of DON increased towards warmer latitudes, and temperature was the main driver of these observations.

4. Ocean warming will increase the nitrogen demand of *Z. marina* and this demand may be met by an increasing uptake of organic nitrogen forms. This indicates that *Z. marina*, and probably other seagrass species, can be winners under global change as nitrogen

uptake capacity will not limit growth driven by increased photosynthetic assimilation of CO_2.

Keywords: DIN uptake, DON uptake, global warming, latitudinal distribution, microbial uptake, seagrasses, temperature, *Zostera marina*

Introduction

Global warming (IPCC, 2018) is affecting the metabolic rates of organisms and the distribution of species, shaping the structure and functioning of marine ecosystems and their trajectories (Walther et al., 2002; Poloczanska et al., 2013). The potential impacts of rising temperatures on organisms and ecosystems are often investigated by short-term experimental temperature manipulation (see Shaver et al., 2000, Rustad et al., 2001 and Aronson & McNulty, 2009 for a review of methods). Controlled temperature experimentation allows separation of temperature effects from other confounding environmental factors that covary with temperature in natural conditions. However, this approach may fail to accurately predict both the magnitude and the direction of species responses due to the transient nature (short-duration) of the experiments and the step increases in experimental temperatures, which are often unrealistic (Rustad, 2008). Testing a species response across a wide latitudinal range is an alternative to address the effects of temperature on organisms because sites along the gradients integrate the geographical climate variation over large temporal scales, thus providing empirical-based predictions of consequences of temperature changes at broader temporal and spatial scales (Fukami & Wardle, 2005; De Frenne, 2013). In the present study, we combined latitudinal gradient analysis with laboratory responses to temperature

manipulation to address how climate warming may affect seagrasses' nutrient acquisition, a key physiological process driving production.

Seagrasses represent one of the most heterogeneous landscape structures of shallow-water marine ecosystems in the world, however, it is predicted that they will be highly impacted by climate change (Bostrom et al., 2006; Chefaoui et al., 2018). Warming may affect seagrasses directly by altering nutrient uptake rates (e.g. nitrogen (N) and phosphorus (P)), or indirectly via a potential acceleration of N mineralisation that may increase seagrass productivity by increasing inorganic nutrient availability in nutrient-limited environments. This process has been well described in terrestrial ecosystems (Rustad et al., 2001; Bai et al., 2013) but is poorly understood in seagrass communities (Duarte et al., 2018).

Seagrasses can take up both dissolved inorganic nitrogen (DIN; nitrate, ammonium) and dissolved organic nitrogen (DON; amino acids, peptides), with a general preference for the uptake of ammonium (NH_4^+) (Alexandre et al., 2011 and references therein), but it is not known how this preference may shift with temperature. In fact, there is little information on whether, or how, seagrass nutrient uptake will respond to global warming (Moore & Short, 2006). A recent study showed that nitrate (NO_3^-) uptake by *Zostera marina* increased by 50% under warming, however, this was undertaken under an unrealistic warming scenario (10 to 18/25 ºC) and exposure to a high nitrate concentration (100 µM; Kaldy, 2014). In terrestrial habitats, it is expected that increased soil temperature will increase plant uptake of N and P in species from warm habitats more than in those from colder environments (BassiriRad, 2000), but warming is also expected to increase plant N and P uptake in arctic species (Jonasson et al., 1999). The few available data also indicate that increased soil temperature elicits a differential effect on the uptake of ammonium versus nitrate, i.e. the ratio of ammonium to nitrate

104 uptake will consistently decrease with increasing temperature in various plant species
105 (BassiriRad, 2000). Potential future changes in the availability of the different N forms
106 in the sediment and water column alongside changes in seagrass N preference under
107 global warming might shift seagrasses' competitive interactions with other species.
108 N availability is one of the major factors limiting primary productivity of seagrasses,
109 particularly in oligotrophic environments where nutrient concentrations are very low
110 (e.g. Agawin et al., 1996; Alcoverro et al., 1997). Seagrasses are well adapted to these
111 conditions, being highly efficient at taking up ephemeral pulses of dissolved inorganic
112 nitrogen (DIN) (Alexandre et al., 2017). On the other hand, the uptake of dissolved
113 organic nitrogen (DON) by seagrasses has been largely overlooked, even though this
114 represents a large component of the total dissolved N pool in coastal waters and is a
115 precursor to DIN formation (Sharp, 1983, 2002; Bronk et al., 2007). In fact, seagrasses
116 can take up N from both simple (amino acids and urea) and complex (peptides) organic
117 substrates at ecologically relevant rates (Vonk et al., 2008; Van Engeland et al., 2011;
118 Alexandre et al., 2015), suggesting that DON may be an important N source for these
119 plants.
120 *Z. marina* is the most abundant seagrass species in the northern hemisphere spreading
121 over a wide latitudinal range, from warm subtropical to arctic regions (Cabello-Pasini et
122 al., 2003; Green & Short 2003). The use of DON by this species was studied at the
123 southern limit of its geographical distribution range, in Ria Formosa lagoon, south
124 Portugal (Alexandre et al., 2015). In this coastal lagoon, DON forms a significant pool
125 of bioavailable N in the water column and in sediments (~ 60 % of total soluble N) and
126 the uptake of DON (as amino acids and peptides) by *Z. marina* represented a significant
127 fraction (~ 30 %) of the total N taken up by the species. However, it is not known how
128 widespread is the use of DON by *Z. marina* and how it may vary along the species'

latitudinal range. Latitude may be relevant as the availability of DON is expected to increase at higher and colder latitudes as DIN regeneration from the mineralisation of organic matter decreases due to decreasing rates of microbial N mineralisation (Chapin et al., 1993; Mozdzer et al., 2014). This trend has been reported in coastal saltmarshes colonized by *Spartina alterniflora* along a latitudinal distribution range (30 - 44 ºN) (Mozdzer et al., 2014). In boreal forests, the low mineralisation rates result in one order of magnitude higher concentrations of DON than those of DIN (Näsholm et al., 2009). Further, in arctic ecosystems the ratios of primary production to N mineralisation are high and consequently arctic vascular plants use amino acid and peptidic N to avoid N limitation derived from low DIN regeneration (Hill et al., 2011). It is also energetically more favourable to take up DON in comparison to DIN as the compounds taken up can be directly used in cell metabolism.

This study assesses how global warming may affect the N uptake rates by the globally widespread seagrass *Z. marina*. Specically we tested the following questions: 1) What is the availability of DON relative to DIN in *Z. marina* meadows along a latitudinal gradient?, 2) What is the latitudinal variation of the uptake rates of DIN and DON by *Z. marina* plant parts (leaves and roots)?, 3) What is the latitudinal variation of the contribution of DIN and DON to the total N budget of *Z. marina*?, 4) What is the latitudinal variation of the microbial uptake of DON and 5) Is temperature the main driver of the latitudinal variations? Laboratory experiments will allow to assess if the latitudinal observations are mainly driven by temperature and consequently what will be the response of *Z. marina* meadows N cycling to global warming.

Methods

Sampling sites

The latitudinal variation of *Zostera marina* uptake rates were assessed in the summer of 2014, when seagrass productivity is highest, in plants collected from meadows located along the species latitudinal distribution range in Europe: a high-latitude meadow located in Alftafjordur, Iceland (64°59'N; 22°36'W), a mid-latitude meadow located in Porthdinllaen, United Kingdom (52°56'N, 4°33'W), and a lower-latitude meadow located in Ria Formosa lagoon, Portugal (36°58'N, 8°02'W) (see Fig. S1 in Supporting Information). Iceland (high-latitude) and Portugal (lower-latitude) represent the northern and southernmost limits of the species distribution, respectively, and United Kingdom (mid-latitude) an intermediate point. The seagrass uptake rates along the latitudinal gradient was assessed by measuring uptake rates of each meadow at the local mean annual temperature. These were combined with temperature manipulation experiments with plants collected at the intermediate site within the distribution (UK), to isolate the temperature effects from other environmental variables. Plant biomass and N availability at each site were used to calculate the contribution of DIN and DON to the total N budget of *Z. marina*.

Samples (n = 5) of seawater (20 mL each) and sediment porewater were collected during low tide (approximate water height of 20 cm) at each meadow to determine the concentration of inorganic (ammonium and nitrate) and organic nitrogen (free amino acids and peptides), as well as total soluble nitrogen (TN). Seawater samples were filtered (Whatman cellulose acetate filters, 0.45 μm pore size) and stored at -20 ºC until analysis. A total of four sediment cores (3 cm diameter, 5 cm depth) were pooled for each porewater sample (~20 mL) to reduce the effect of nutrient heterogeneity. The four pooled cores were randomly collected within the meadow at an approximate distance of 10 cm, and the different groups of pooled cores were collected at a distance of at least 2

m. The sediment samples were centrifuged (2300 *g*, 15 min at 4 ºC) and the supernatants were filtered (Whatman cellulose acetate filters, 0.45 µm pore size) and frozen until analysis.

Shoot density (no. shoots m^{-2}) and areal biomass (g dry weight m^{-2}) were also determined for each site by counting the number of shoots and weighing the dried plants (48 h at 60 ºC) collected within a sampling quadrat (20 cm x 20 cm, n = 3). The quadrats were haphazardly tossed inside the meadow, ensuring distances higher than 20 cm between different tossings.

Plant collection and acclimation

Z. marina plants were collected during low tide from the meadows and cleaned of adhering sediment, avoiding damage of the root hairs, and epiphytes removed from the leaves by gentle hand scraping. The plants were transported in seawater collected at the sampling site to the laboratory, where they were acclimated for 3 days at local summer conditions of light and mean annual temperatures (Marine and Freshwater Research Institute; climate-data.org; Global Sea Temperature database). In Iceland, plants were acclimated at 8 ºC with a 20:4 h light:dark cycle; in the UK, plants were acclimated at 12 ºC with a 18:6 h light:dark cycle and in Portugal at 17 ºC with a 16:8 h light:dark cycle.

Z. marina nitrogen uptake experiments

Uptake rates of inorganic (ammonium and nitrate) and organic (alanine and trialanine) N were determined by incubating whole plants fully immersed in 300 mL of N-free artificial seawater (0.2 µm filtered water, salinity of 35, pH 8.2) containing either $^{15}NH_4Cl$, $^{15}KNO_3$ or $^{13}C_3H_7{}^{15}NO_2$ (L-alanine) at concentrations of 5, 25, 50 and 100 µM

204 or $^{13}C_9H_{17}{}^{15}N_3O_4$ (L-trialanine) at concentrations of 5, 15 and 50 μM (atom % = 98,
205 alanine obtained from Cambridge Isotope Laboratories; trialanine obtained from CK
206 Gas Products, Hook, UK). Above and belowground biomass of incubated tissues are
207 given in Table S1. Alanine was selected because it is one of the amino acids with higher
208 rates of occurrence in the proteins of all types of organisms and therefore is important in
209 N cycling. Trialanine was chosen because of its ecological significance as it represents a
210 common structure that is released by protein hydrolysis. All experiments were
211 performed at constant light (≈ 100 μmol quanta m^{-2} s^{-1}) for 0.5 h and at each site-
212 specific acclimation temperature. Incubations with the four N sources at the different
213 nutrient concentrations were done simultaneously whereas replicates (n = 3) were done
214 sequentially to overcome feasibility constrains derived form the setup of a large number
215 of incubations chambers necessary to test all treatments simultaneously (N sources and
216 N concentrations, n = 16 chambers).
217 At the end of uptake experiments, the leaves were immediately separated from the
218 rhizomes and roots. Tissues were rinsed with deionised water to remove adhering salt
219 and isotopic labels before being dried (60 ºC, 48 h), and reduced to a fine powder for
220 analysis of the atom % of ^{15}N and total N content.
221
222 Microbial DON uptake experiments
223 At each meadow, seawater samples and sediment cores of 3 cm diameter and 5 cm
224 depth were collected (n = 3), placed in 50 mL polypropylene centrifuge tubes, sealed
225 immediately and transported on ice to the laboratory. Sediment samples were
226 acclimated for three days to the site-specific temperature (8, 12 and 17º C in Iceland,
227 UK and Portugal, respectively). Sediment sub-samples of 1 g of fresh weight were then
228 placed in 1.5 mL polypropylene tubes. The water content of natural sediments (~ 0.5

mL g^{-1} fresh weight) was previously determined by drying at 105 ºC for 24 h and used to calculate the amount of seawater needed to add to the sediment in order to obtain a final incubation volume of 1 mL after sediment centrifugation. Artificial seawater with a salinity equal to the natural seawater of each site was purged with N_2 to remove oxygen and added to the sediment tubes to reach the final incubation volume of 1 mL of water content in each tube. Microbial uptake rates of amino acids and peptides were determined by adding ^{14}C-labelled alanine or trialanine solutions (20 µL; 2.8 kBq mL^{-1} seawater or g^{-1} soil; American Radiolabeled Chemicals, St Louis, MO, USA) to the seawater and sediment sub-samples at a range of concentrations (5, 25, 50 and 100 µM for alanine and 5, 15 and 50 µM for trialanine). Samples from Iceland, UK and Portugal were incubated respectively at 8, 12 and 17 ºC for 0.5 h under vigorous shaking. After incubation, samples were centrifuged (18 000 *g*, 10 min) and the supernatant recovered. Subsequently, 0.2 mL of HCl (1 M) was added to release any $H^{14}CO_3^-$ trapped in solution (Brailsford et al., 2019) and the ^{14}C activity in the supernatant (0.3 mL) determined on a Wallac 1404 liquid scintillation counter with automatic quench correction (Wallac EG&G, Milton Keynes, UK) after addition of 4 mL of HiSafe3 liquid scintillation cocktail (Perkin Elmer Corp., Waltham, MA). Unfortunately, it was not possible to measure the microbial uptake of alanine and trialanine in the water in the absence of sediment as the ^{14}C activity measured at the end of the experiment was never lower than the initial ^{14}C activity, even when the incubation time was extended (i.e. no measureable uptake).

Effects of temperature on N uptake

To evaluate the effect of seawater temperature on the uptake rates of *Z. marina* (DIN and DON) and microbes (DON), plants and sediment cores (3 cm diameter, 5 cm depth)

were collected from the mid-latitude site (UK) and were incubated at the latitudinal range of temperatures of 8, 12 and 17 ºC. The rationale for this was that mid-latitude plants are within the range of mean annual temperatures (8, 12 and 17 ºC) of the three latitudes studied, i.e. the minimum (8 ºC) and maximum (17 ºC) temperatures to which mid-latitude plants are exposed coincide with the mean annual temperature of plants from the high-latitude site in Iceland (8 ºC) and from the lower-latitude site in Portugal (17 ºC). Plants and sediments were acclimated for three days to the experimental conditions of light (~ 100 µmol quanta m^{-2} s^{-1}) and temperature under a 18:6 h light:dark cycle. The uptake rates of inorganic (ammonium and nitrate) and organic (alanine and trialanine) nitrogen by *Z. marina* and the microbial uptake of DON were determined as described above. The average leaf biomass per incubated shoot was 0.24 g dry weight, whereas the average belowground biomass was 0.14 g dry weight.

Sample analysis

Concentrations of dissolved inorganic nitrogen (DIN) were determined colorimetrically in a loop-flow analyzer (µMAC-1000 Systea, Agnani, Italy). Samples for determination of total free amino acids and amino acids bound in short peptides were passed through a 1 kDa ultrafiltration membrane (Millipore, Billerica, Massachussets, USA). Amino acid N was determined fluorometrically before and after hydrolysis in 6 M HCl at 105 °C for 16 h under N_2 using glycine as a standard (Jones et al., 2002). Glycine was used because it is the most predominant amino acid and its relative fluorescent intensity is similar to that of other dominant amino acids (Parsons et al. 1984). Peptide N was determined as the difference between the free and total amino acid pools, determined before and after hydrolysis, respectively. Total nitrogen (TN) was determined after combustion to nitrogen monoxide by chemiluminescence using a TOC-V-TN analyser (Shimadzu,

Kyoto, Japan). TN content and atom % ^{15}N of dried tissues were determined using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Precision of the $\delta^{15}N$ analysis was higher than 0.03 ‰. Leaf and root ^{15}N background level was measured on five replicate samples.

Data analysis

The N uptake rates of *Z. marina* were expressed in μmol g^{-1} dry weight h^{-1}. ^{15}N enrichment (%) of tissues after incubation was calculated by subtracting the post-incubation ^{15}N levels from the initial background levels, multiplied by the total N content (g) in the tissue and divided by its dry weight (g dry weight). Uptake rates were plotted against substrate concentration (μM) and the uptake kinetic parameters were derived using the Michaelis-Menten kinetic model

$$V = (V_{max} \times S) / (K_m + S)$$

where V is uptake rate (μmol g^{-1} dry weight h^{-1}), V_{max} is maximum uptake rate (μmol g^{-1} DW h^{-1}), S is substrate concentration (μM) and K_m is the half-saturation constant (μM). Data not displaying saturation kinetics were fitted to a linear regression model (V = uptake rate, S = substrate concentration).

Whole-plant nitrogen budgets per ground area (μmol m^{-2} h^{-1}) of *Z. marina* along the latitudinal gradient and at each experimental temperature were estimated by multiplying the N uptake rates (μmol g^{-1} dry weight h^{-1}) of the plants at the ambient nutrient concentrations (μM) measured in each site by the areal biomass (g^{-1} dry weight m^{-2}). The DIN and DON contribution to the total N acquisition by the leaves and roots were calculated by dividing the sum of the acquisition of ammonium and nitrate, or amino acids and peptides, of each plant part by the total plant N acquisition.

Microbial DON uptake rates were expressed in nmol mL^{-1} h^{-1}. ^{14}C microbial uptake of alanine and trialanine was calculated based on the percentage of ^{14}C-labelled solution taken up by the microbes, obtained from the proportion of initial ^{14}C activity remaining in solution after incubation.

Differences in ambient concentrations between study sites were tested for each N source separately using one-way analysis of variance (ANOVA). The effects of latitude / nutrient concentration and temperature / nutrient concentration on N uptake rates by *Z. marina* were tested using two-way ANOVAs for each N source and plant part. All other differences in *Z. marina* uptake rates were tested using the non-parametric Kruskal-Wallis test because the assumption of data normality was not met even after data transformation.

The effects of latitude / trialanine concentration and of temperature / alanine concentration on microbial uptake rates were tested using two-way ANOVA. The effects of latitude / alanine concentration and of temperature / trialanine concentration on microbial uptake rates were tested with the non-parametric Kruskal-Wallis test because the ANOVA assumptions of data normality and equal variance were not met even after data transformation. Data were square root transformed when normality was not verified. All tests were performed at a level of significance of $p < 0.05$.

Results

Concentration of inorganic and organic N

The concentrations of the different forms of soluble N were generally much higher in the sediment porewater than in the water column at all sites (Table 1). The nutrient concentrations in the water column did not vary significantly among sites except for

329 ammonium and amino acids, which were higher in Portugal and in the UK, respectively.
330 The concentration of ammonium in the sediment porewater was not significantly
331 different among the study sites, whereas the concentration of all other N sources varied
332 significantly but with no consistent latitudinal pattern.
333 Amino acids and peptides, a fraction of the dissolved organic nitrogen (DON) pool,
334 constituted an important component of the total nitrogen available in both the sediment
335 and water column of the studied sites, representing 53%, 29% and 43% of the total
336 nitrogen in the sediment and 52%, 13% and 41% in the water, respectively in Iceland,
337 UK and Portugal. No latitudinal consistency was found.
338
339 Plant density and biomass
340 Shoot density in Iceland (1483 ± 379 shoots m^{-2}) was much higher than in the UK (533
341 ± 113 shoots m^{-2}) and Portugal (408 ± 52 shoots m^{-2}) but the aboveground biomass was
342 lower at the northern limit of the species distribution (44 ± 11 g dry weight m^{-2})
343 compared to the UK (185 ± 12 g dry weight m^{-2}) and Portugal (130 ± 9 g DW m^{-2}) due
344 to the smaller size of the leaves (leaf length: 15 ± 4 cm in Iceland, 37 ± 10 cm in the UK
345 and 32 ± 9 cm in Portugal). Below-ground biomass was conservative: in Iceland it was
346 55 ± 5 g dry weight m^{-2}, in the UK it was 56 ± 12 g dry weight m^{-2} and in Portugal it
347 was 55 ± 5 g dry weight m^{-2}. *Z. marina* from Iceland showed higher concentrations of
348 nitrogen in the leaf tissues (2.35 ± 0.2 % dry weight) compared to plants from the UK
349 and Portugal (1.65 ± 0.4 and 1.83 ± 0.2 % DW, respectively).
350
351 N uptake by *Zostera marina*
352 Overall, the DIN and DON uptake rates by *Z. marina* was higher towards the equator,
353 and reduced in the poleward direction, particularly at higher substrate concentrations

(Fig. 1, Table S2). *Z. marina* growing at the southern limit of its distribution (Portugal) showed significantly higher uptake rates compared to plants growing further north except for the uptake of ammonium through the roots, which was similar between latitudes at all nutrient concentrations (Fig. 1e, Table S2). Differences in the uptake rates between latitudes at the lowest N concentration (5 μM) were only detected for organic N sources (alanine and trialanine). Maximum nitrogen (inorganic and alanine) uptake rates (V_{max}) consistently increased with decreasing latitude, with a 2 to 4-fold decrease from Portugal to Iceland (Table S3). The latitudinal effects on nutrient affinity (α) and on the half-saturation constant (K_m) were not as clear. The increase of V_{max} with decreasing latitude is mostly caused by temperature, as indicated by the temperature experiment, where V_{max} increased significantly with temperature (Table S4). The results of the two-way analysis of variance examining the effects of temperature (T) and nutrient concentration (N) on the nitrogen uptake rates of *Zostera marina* support that the latitudinal pattern observed is caused by temperature as higher temperatures resulted in higher N uptake (Fig. 2, Table S5).

Whole-plant N budget

The leaf, root and whole-plant N budgets of *Z. marina* increased with decreasing latitude both for DIN and DON (Fig. 3a, Table S6). Root uptake was more important for *Z. marina* nitrogen budget than leaf uptake as it contributed 93%, 72% and 70% to the whole-plant budget, respectively in Iceland, UK and Portugal (Fig. 3b, Table S6). These values also show that root contribution decreases with decreasing latitude. Overall, the uptake of DON by *Z. marina* contributed about one third to the whole-plant nitrogen budget, showing that this N form is ecologically relevant throughout the latitudinal gradient. Ammonium represented the largest contributor to the total DIN budget of the

379 species at all sites, particularly via root uptake from the sediment (66% in Iceland, 44%
380 in UK and 41% in Portugal, Table S6). The contribution of amino acids and peptides
381 via root uptake (26 % in Iceland, 27% in UK and 24% in Portugal) was smaller
382 compared to ammonium but much higher than that of nitrate (2, 0.5 and 5%,
383 respectively).
384 The temperature experiments showed a general pattern of increasing DIN and DON
385 budgets towards warmer sites, except for the DIN and DON of leaves (Fig. 3c, Table
386 S7). The relative contributions of DIN and DON to the whole-plant budgets did not
387 vary with latitude, but the relative contribution of DON to the whole plant N budget
388 increased with temperature, as opposed to DIN (Fig. 3d, Table S7).
389
390 Microbial DON uptake
391 The sediment microbial uptake of alanine did not change significantly among sites ($H = 4.491$, $p = 0.106$), as opposed to the uptake of trialanine that increased with decreasing
393 latitude (Fig. 4a and b). Despite the lack of a significant latitudinal variation of alanine,
394 the temperature experiments showed significant effects on the microbial uptake rates of
395 alanine at the highest nutrient concentration (100 µM) ($F = 20.46$, $P < 0.001$) (Fig. 4c).
396 The microbial uptake of trialanine increased significantly from 8 ºC to 12 ºC and 17 ºC
397 ($H = 6.423$, $P = 0.04$) (Fig. 4d).
398
399 Discussion
400
401 Our study showed that the uptake rates of inorganic and organic N by both leaves and
402 roots and the total meadow uptake under local conditions (N budget) of the globally
403 widespread seagrass *Zostera marina* increased from northern to southern sites. The

hypothesis that temperature is the main driver of this latitudinal trend was supported by the temperature experiments.

N uptake rates and N budget of *Z. marina*

For all N sources, the uptake rates and the uptake kinetics of *Z. marina* increased with temperature as reported for the uptake rates of ammonium and glycine by the Arctic saltmarsh grass *Pucinellia phryganodes* (Henry & Jefferies, 2003). Kaldy (2014) is, to our knowledge, the only other report that has experimentally tested the effects of temperature on the acquisition of nitrogen by *Z. marina*. This study showed a 50 % increase in nitrate uptake as the temperature increased from 10 to 18/25 ºC in plants exposed to elevated nitrate concentrations (100 µM). Indirect indications of temperature effects on the acquisition of nitrogen by seagrasses have been presented in studies evaluating the seasonal variation of N uptake rates (Lee & Dunton, 1999; Hasegawa et al., 2005) or the influence of season and temperature on the activity of enzymes involved in the nitrogen metabolism, such as glutamine synthetase (Kraemer & Mazzella, 1999) and nitrate reductase (Alexandre et al., 2004). In these studies, the uptake rates and the enzymatic activities generally increased with increasing temperature. Despite the greater amount of bioavailable DON relative to DIN in all studied sites, the uptake of DIN represented the largest fraction of the total N budget of *Z. marina* across its latitudinal distribution. This was due to the high uptake of ammonium (54 - 68 % of the total N uptake), which is the preferential nitrogen source of *Z. marina* (Short & McRoy, 1984; Hemminga et al., 1994; Alexandre et al., 2015). Even though the rate of nitrogen uptake through the leaves was generally higher than through the roots at similar nutrient concentrations, the overall contribution of root uptake to the total N budget of *Z. marina* (70 - 94 %) was much higher than that of

leaves (7 - 30 %) because the concentrations of the different nitrogen sources in the sediment porewater were several-fold higher than in the water column. This relative contribution of the different plant parts contrasts with other reports showing an equal contribution of leaves and roots to the total annual N acquisition by *Z. marina* (Pedersen & Borum, 1993; Lee & Dunton, 1999), but their calculations accounted only for inorganic nitrogen. Amino acids and oligopeptides are a relevant source of nitrogen for *Z. marina*, particularly through root uptake. The average contribution of amino acids (12 %) and peptides (14 %) via root uptake exceeded that of nitrate (2 %) by several-fold at the extremes of the latitudinal gradient. This result supports the relevant role of organic nitrogen as a complementary N source for *Z. marina*, previously reported by Alexandre et al. (2015).

In contrast to our initial hypothesis, the pool of bioavailable DON did not increase with latitude driven by lower microbial N mineralisation and this was not reflected in higher DON uptake by *Z. marina*, as observed for arctic and antarctic vascular plants (Chapin et al., 1993, Hill et al., 2011). Both the uptake of DON by *Z. marina* and the DON contribution to the whole-plant N budget increased towards lower latitude and this trend was driven by higher temperature. At high latitude, the lower contribution of DON to the total N budget of *Z. marina* is determined by the plants' lower DON uptake rates rather than by a lower availability of DON sources.

Microbial DON uptake

The microbial uptake of DON in *Z. marina* sediments increased towards southern latitudes and this was driven by temperature. DON uptake is ecologically relevant as amino acids and peptides accounted for a significant pool of bioavailable nitrogen in *Z. marina* meadows, particularly in the sediments (30 to 50 % of the total N). The

454 observed latitudinal pattern contrasts with that reported by Mozdzer et al. (2014), who
455 found significant increases in sediment DON availability with increasing latitude in
456 *Spartina alterniflora* saltmarshes, which coincided with a significant decrease in
457 microbial DON uptake.
458 To the best of our knowledge, this was the first report of the latitudinal effects of
459 temperature on the DON uptake of both micobes and seagrasses, which is relevant to
460 understand their competition for these nitrogen sources. Dissolved organic nitrogen
461 serves both as a direct source of nitrogen for *Z. marina* and as a mineralisation substrate
462 for its associated microbial community, and both of their uptake rates increase with
463 temperature. Seagrasses in general and *Z. marina* in particular may have a competitive
464 advantage over microbes for DON uptake because they also acquire nutrients through
465 the leaves from the water column, where microbial uptake of alanine and trialanine was
466 very low, according to the lower ^{14}C uptake when compared to the sediment.
467 The northern distribution limits of *Z. marina* are warming twice as fast as the rest of the
468 globe (IPCC, 2018) creating the conditions for its expansion towards the pole. This
469 warming trend will increase the nitrogen demand of *Z. marina* that may be met by an
470 increasing uptake of organic nitrogen forms. The competition with microbes for this
471 nutrient source is also expected to increase as warming will also enhance the microbial
472 uptake of DON. Our results highlight that *Z. marina*, and probably other seagrass
473 species, can be winners under global change conditions of increased temperature and
474 CO_2. Many reports have shown that higher CO_2 levels will increase the photosynthetic
475 production of seagrasses (Jiang et al., 2010, Alexandre et al., 2012, Ow et al., 2015),
476 which may result in higher growth that will not be limited by the plants' nitrogen uptake
477 capacity.
478

Acknowledgements

We thank J. Svavarsson and E. Olafsson for their support at the University of Iceland, C.B. de los Santos for sharing the geographical coordinates of the site in Iceland, and S. Chesworth for the analysis of total nitrogen at Bangor University. We also thank two anonymous reviewers for their constructive comments on the manuscript. A.A. was supported by a postdoctoral fellowship from the Portuguese Foundation for Science and Technology (FCT, SFRH/BPD/91629/2012). This study received Portuguese national funds from FCT - Foundation for Science and Technology through project UIDB/04326/2020, and in the ambit of the contract program DL 57/2016/CP1361/CT0001.

Authors contribution

A.A. and R.S. conceived and designed the study. A.A., R.Q. and P.W.H. carried out the experimental work. A.A., P.W.H., D.J. and R.S. analysed the data. A.A. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

Data are available from the Mendeley Data under doi: 10.17632/r4m88s8m8w.1 (Alexandre and Santos 2020 Zm N uptake latitude and temperature).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Table S1. Average aboveground and belowground biomass of *Zostera marina* incubated in each site along the latitudinal distribution gradient.

Table S2. Nitrogen uptake kinetic parameters of *Zostera marina* along the species' latitudinal distribution gradient.

Table S3. Results of the two-way ANOVA examining the effects of latitude and nutrient concentration on the nitrogen uptake rates of *Zostera marina*.

726 Table S4. Nitrogen uptake kinetic parameters of *Zostera marina* at three different
727 temperatures.
728 Table S5. Results of the two-way ANOVA examining the effects of temperature and
729 nutrient concentration on the nitrogen uptake rates of *Zostera marina*.
730 Table S6. Whole-plant nitrogen budget of *Zostera marina* along the species latitudinal
731 distribution range in Europe.
732 Table S7. Whole-plant nitrogen budget of *Zostera marina* at three different
733 temperatures.
734 Figure S1. Aspect of the *Zostera marina* meadows in Iceland, United Kingdom and
735 Portugal.
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743 Table 1. Concentration (µM) of the different nitrogen sources (ammonium, nitrate, free
744 amino acids, peptides and total nitrogen) in the water column and sediment porewater in
745 the study sites (Iceland, United Kingdom and Portugal). Values are mean ± S.D. (n = 5).
746 Levels of significance of the statistical tests are indicated as (*) $P < 0.05$, (**) $P < 0.01$,
747 (***) $P < 0.001$, (^{ns}) not significant. Different letters indicate significant differences.
748 Peptide values in italic are not reliable as they are higher than total nitrogen and higher
749 than sediment values (see text in Supporting Information).
750

	Iceland	United Kingdom	Portugal	Test statistic
Water column				
Ammonium	0.18 ± 0.19[a]	0.77 ± 0.36[a]	1.35 ± 1.06[b]	H = 7.322*
Nitrate	0.43 ± 0.68	0.14 ± 0.06	3.12 ± 1.89	H = 4.842[ns]
Amino acids	0.29 ± 0.10[a]	0.67 ± 0.30[b]	0.29 ± 0.11[a]	F = 6.483*
Peptides (estimated)[§]	2.50 ± 1.83	1.03 ± 0.49	4.82 ± 4.04	H = 5.700[ns]
Total nitrogen	5.33 ± 3.90	12.86 ± 6.14	12.36 ± 10.35	F = 2.784[ns]
Sediment				
Ammonium	20.87 ± 8.49	20.03 ± 8.98	30.17 ± 6.75	F = 1.769[ns]
Nitrate	0.66 ± 0.55[a]	0.54 ± 0.16[a]	15.08 ± 5.09[b]	F = 26.201***
Amino acids	7.83 ± 4.25[a]	62.25 ± 20.06[b]	6.72 ± 2.12[a]	H = 7.385*
Peptides	42.23 ± 16.43[ab]	17.70 ± 13.33[a]	50.59 ± 12.59[b]	F = 5.081*
Total nitrogen	93.93 ± 29.35[a]	278.73 ± 102.57[b]	134.72 ± 33.49[ab]	H = 9.118***

751 [§]Concentrations estimated from the percentage of peptides relative to the total soluble
752 nitrogen in the sediment porewater (see text).
753

754 Figure 1. Uptake rates (μmol g^{-1} dry weight h^{-1}) of the different nitrogen sources by
755 leaves and roots of *Zostera marina* as a function of concentration (μM) along the
756 species latitudinal distribution gradient (Iceland, United Kingdom and Portugal):
757 ammonium (a), nitrate (b), alanine (c) and trialanine (d) by the leaves; ammonium (e),
758 nitrate (f), alanine (g) and trialanine (h) by the roots. Values are mean ± S.D. (n = 3).
759 DW = dry weight.
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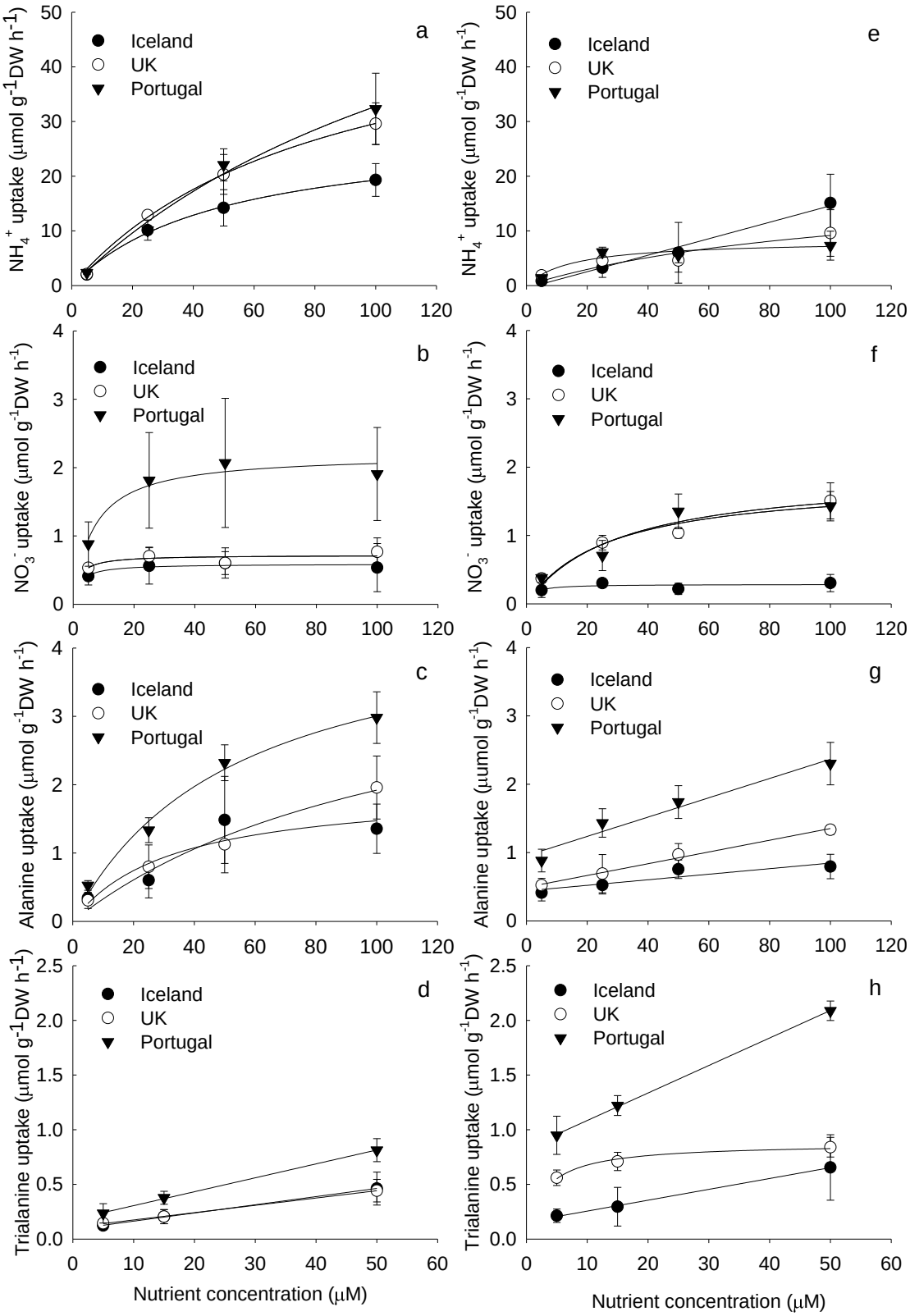

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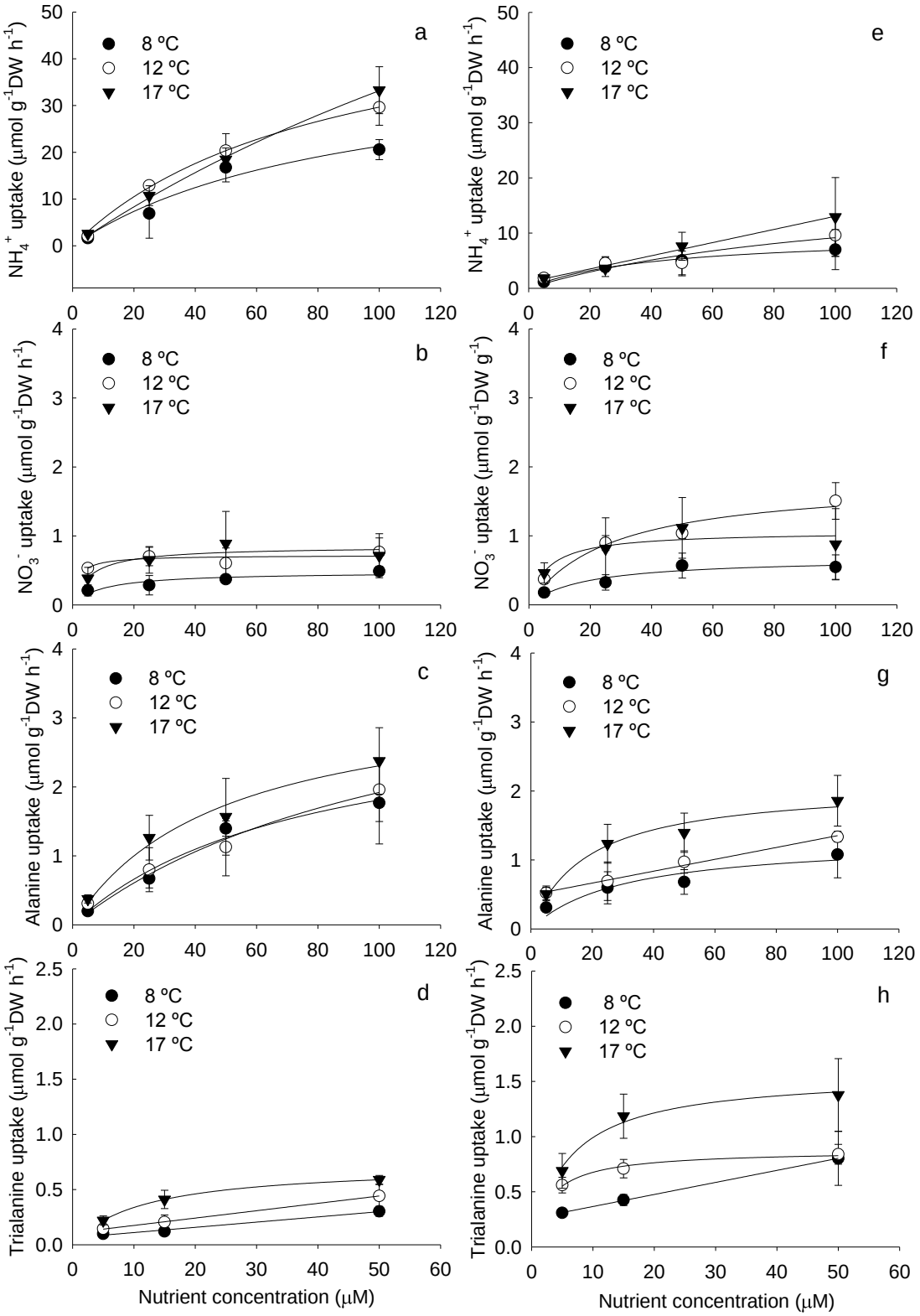
768 Figure 2. Uptake rates (μmol g^{-1} dry weight h^{-1}) of the different nitrogen sources by
769 leaves and roots of *Zostera marina* as a function of concentration (μM) at three
770 incubation temperatures (8, 12 and 17 ºC): ammonium (a), nitrate (b), alanine (c) and
771 trialanine (d) by the leaves; ammonium (e), nitrate (f), alanine (g) and trialanine (h) by
772 the roots. Values are mean ± S.D. (n = 3). DW = dry weight.



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779 Figure 3. Whole-plant nitrogen budget of *Zostera marina* along the species latitudinal
780 distribution range in Europe (Iceland, United Kingdom and Portugal) as µmol m^{-2} h^{-1} (a)
781 and % contribution (b), and the experimental effects of temperature on the whole-plant
782 nitrogen budget as µmol m^{-2} h^{-1} (c) and % contribution (d).
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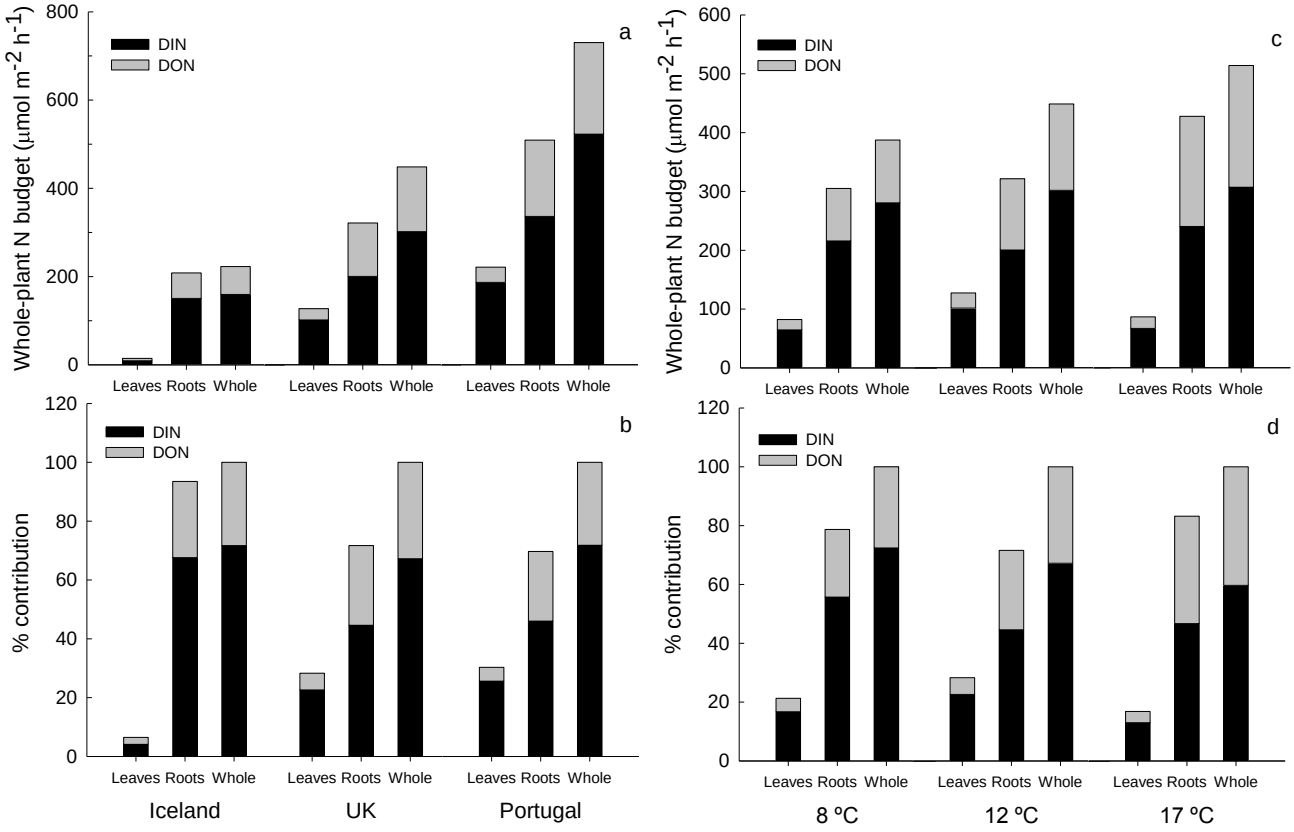

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798 Figure 4. Microbial uptake (nmol mL^{-1} h^{-1}) of alanine (a) and trialanine (b) as a function
799 of substrate concentration (µM) in *Zostera marina* meadows at three sites (Iceland,
800 United Kingdom and Portugal) along the species latitudinal distribution gradient, and
801 the experimental effects of temperature on the microbial uptake of alanine (c) and
802 trialanine (d) . Values are mean ± SD (n = 3). Significant differences are indicated by
803 different letters. ($P < 0.05$).
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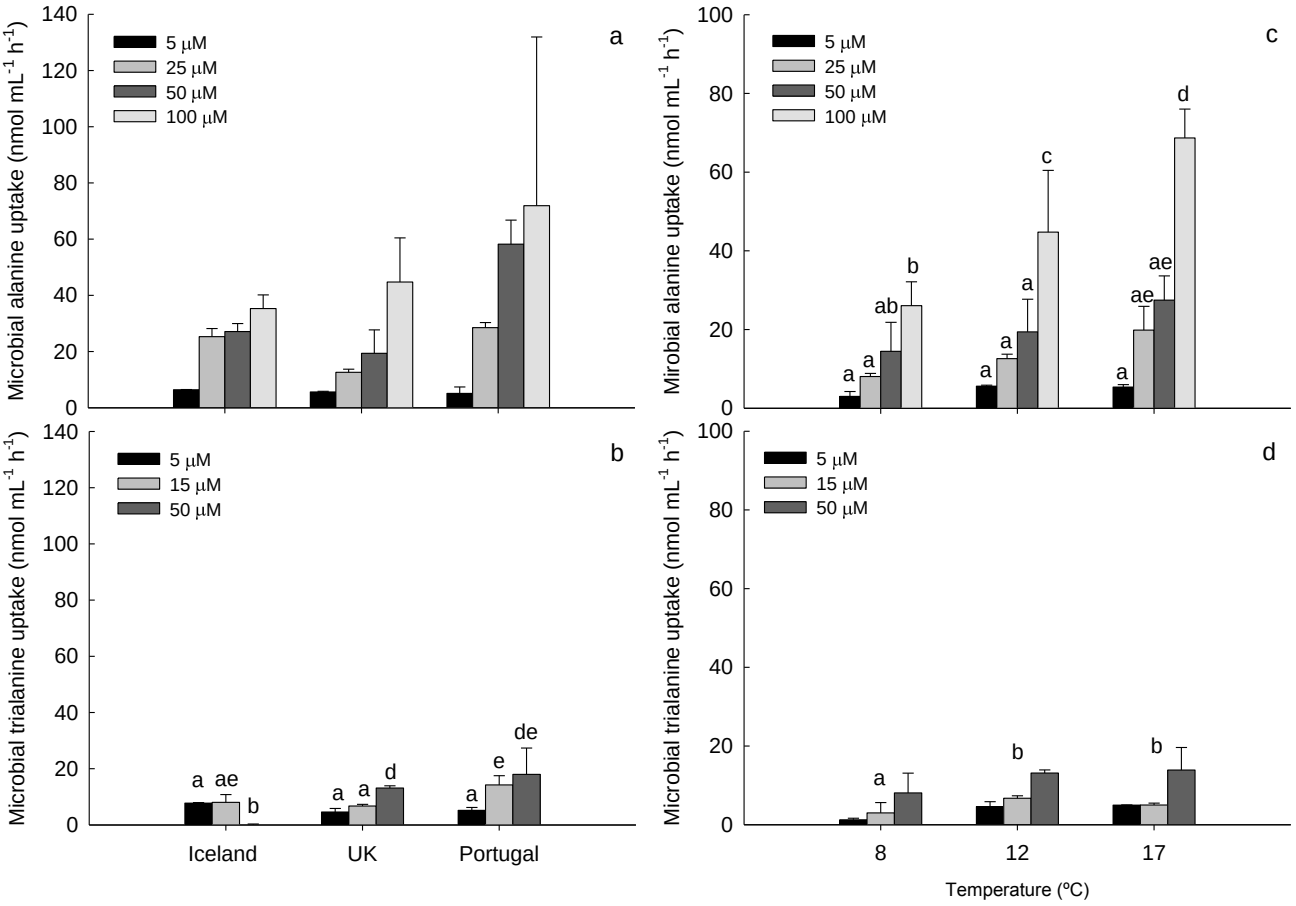

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SUPPORTING INFORMATION

Supplementary Methods

Z. marina nitrogen uptake experiments

During incubations, plant shoots were loose in the incubation chambers and naturally gained a relatively vertical alignment, with their roots pointing towards the bottom of the chamber and the leaves to the surface. The media were constantly stirred during incubation to decrease the thickness of the boundary layer at the leaf/root surface and to ensure a homogeneous distribution of the isotopic labels and that leaf surfaces received similar amounts of photosynthetic active radiation. The authors acknowledge that, in this experiment, belowground plant parts were incubated in oxygenated conditions, which is in contrast with the anoxic natural environment. However, preliminary experiments showed no effect of rhizosphere oxygenation on the ammonium uptake rates of leaves and roots, as was also reported for the sister species *Z. noltei* (Alexandre et al., 2010; Alexandre et al., 2011).

Because no significant internal translocation of incorporated ^{15}N between leaves and roots is expected within the chosen experimental incubation time (Vonk et al., 2008; Alexandre et al., 2011), we assumed that the ^{15}N content determined in leaf and root tissues after incubation represents only the amount of N that was taken up by the respective plant parts. The removal of a substantial part of the microbial community from the incubation media (artificial seawater filtered through 0.2 µm) and the short incubation time (0.5 h) restricted exogenous DON mineralisation during the incubations (Sharkey, 2007; Bauwe et al., 2010). Therefore based on these many previous studies,

we assume that most, if not all, of the amino acids and peptides were taken up by the leaves and roots of *Z. marina* without prior cleavage.

Sample analysis

The concentration of ammonium was determined using the hypochlorite/indophenol method (detection and quantification limits = 0.03 and 0.07 μM), whereas nitrate was determined by the cadmium reduction method (detection and quantification limits = 0.06 and 0.11 μM). Amino acid N was determined according to Jones et al. (2002). Briefly, 20 μL of sample or standard and 200 μL of working reagent (5 mL of OPAME concentrate + 200 mL of borate buffer 0.02 M pH 9.5) were combined and read after 1 min on a LS-5B Fluorometer (Perkin Elmer Corp., Boston, MA) with an excitation wavelength of 340 nm and an emission wavelength of 450 nm. The OPAME reagent was obtained by dissolving 50 mg of *o*-phthaldialdehyde in 5 mL of methanol and adding 100 μL of β-mercaptoethanol.

Supplementary Results

Concentration of inorganic and organic N

The concentration of peptides in the water column was higher than in the sediment, and exceeded the total N by 3 to 10-fold, an incongruity that was not observed in the sediment porewater (Table 1). We concluded that these values are not reliable, suggesting an unresolved technical issue (e.g. contamination or unknown intereference). To overcome this, water column peptide values were estimated from the percentage of peptides relative to the total soluble nitrogen observed in the sediment porewater (50 % in Iceland, 8 % in the UK and 40 % in Portugal).

N uptake by *Zostera marina*

The uptake of dissolved inorganic nitrogen (DIN) by *Z. marina* generally displayed saturation kinetics whereas the uptake of DON was in most cases best described by a linear model, indicating that saturation was not reached within the range of nutrient concentrations used in the experiment (Fig. 1, Table S2).

The uptake of trialanine from the sediments increased only slightly with nutrient concentration ($F = 4.083$, $p = 0.035$) except for trialanine in Iceland, where values at 50 µM (0.12 nmol mL^{-1} h^{-1}) were much smaller than at 5 µM and 15 µM (7.75 and 8.05 nmol mL^{-1} h^{-1}, respectively) (Fig. 4).

Supplementary Tables

Table S1. Average aboveground (leaves) and belowground (rhizomes + roots) biomass (g dry weight) of *Zostera marina* incubated in the high-, mid- and lower-latitude sites along the latitudinal distribution gradient.

	High-latitude	Mid-latitude	Lower-latitude
Aboveground	0.05	0.24	0.29
Belowground	0.03	0.13	0.22

Table S2. Uptake kinetic parameters of nitrogen uptake by *Zostera marina* plant parts at the three sites (Iceland, United Kingdom and Portugal) along the species' latitudinal distribution gradient. V_{max} = maximum uptake rate (μmol N g^{-1} dry weight h^{-1}); K_m = half-saturation constant (μM); and α = affinity constant (V_{max}/K_m). The coefficient of determination (r^2), level of significance (P) and the standard error of the estimate (values in brackets) are given. Data not displaying saturation kinetics were fitted with a linear regression model (V = uptake rate; S = substrate concentration).

	V_{max}	K_m	α	r^2	P
Ammonium (leaves)					
Iceland	28.91 (2.30)	49.92 (8.73)	0.58	0.996	0.002
United Kingdom	54.63 (5.18)	84.24 (14.42)	0.65	0.997	0.001
Portugal	82.08 (24.48)	150.62 (67.20)	0.55	0.990	0.005
Nitrate (leaves)					
Iceland	0.59 (0.03)	2.05 (0.86)	0.29	0.83	0.087
United Kingdom	0.72 (0.06)	1.75 (1.27)	0.41	0.60	0.224
Portugal	2.19 (0.16)	6.56 (2.30)	0.33	0.94	0.029
Alanine (leaves)					
Iceland	1.95 (0.78)	32.60 (34.34)	0.06	0.82	0.096
United Kingdom	4.19 (1.50)	118.18 (67.95)	0.04	0.97	0.014
Portugal	4.62 (0.63)	53.68 (15.66)	0.09	0.99	0.006
Trialanine (leaves)					
Iceland	V = 0.089+0.007 S	-	-	1.00	0.012
United Kingdom	V = 0.108+0.007 S	-	-	1.00	0.012
Portugal	V = 0.178+0.013 S	-	-	0.99	0.016
	V_{max}	K_m	α	r^2	P
Ammonium (roots)					
Iceland	V = -0.486+0.151 S	-	-	0.99	0.007
United Kingdom	19.39 (14.37)	111.61 (135.66)	0.17	0.87	0.069
Portugal	8.26 (1.58)	15.39 (10.65)	0.54	0.89	0.054
Nitrate (roots)					
Iceland	0.29 (0.04)	2.02 (2.19)	0.14	0.43	0.346
United Kingdom	1.81 (0.30)	27.00 (2.70)	0.07	0.95	0.027
Portugal	1.93 (0.45)	30.37 (19.23)	0.06	0.92	0.042
Alanine (roots)					
Iceland	V = 0.439+0.004 S	-	-	0.82	0.093
United Kingdom	V = 0.493+0.009 S	-	-	0.99	0.004
Portugal	V = 0.954+0.014 S	-	-	0.96	0.022
Trialanine (roots)					
Iceland	V = 0.156+0.010 S	-	-	0.99	0.023
United Kingdom	0.88 (0.03)	2.96 (0.56)	0.30	0.98	0.09
Portugal	V = 0.832+0.025 S	-	-	1.00	0.011

Table S3. Results of the two-way ANOVA examining the effects of latitude (L) and nutrient concentration (N) on the nitrogen uptake rates of *Zostera marina*. Test statistic (F) and levels of significance are indicated as (*) P < 0.05, (**) P < 0.01, (***) P < 0.001, (^{ns}) not significant.

	Latitude (L)	**Nutrient concentration (N)**	**L x N**
Leaves			
Ammonium	$F = 11.87^{***}$	$F = 118.2^{***}$	$F = 3.73^{**}$
Nitrate	$F = 24.48^{***}$	$F = 2.45^{ns}$	$F = 1.15^{ns}$
Alanine	$F = 22.51^{***}$	$F = 45.70^{***}$	$F = 3.16^{*}$
Trialanine	$F = 18.31^{***}$	$F = 55.05^{***}$	$F = 2.49^{ns}$
Roots			
Ammonium	$F = 0.02^{ns}$	$F = 24.33^{***}$	$F = 2.12^{ns}$
Nitrate	$F = 101.73^{**}$	$F = 38.47^{***}$	$F = 7.86^{***}$
Alanine	$F = 84.83^{***}$	$F = 35.18^{***}$	$F = 3.96^{**}$
Trialanine	$F = 118.12^{***}$	$F = 43.46^{***}$	$F = 8.45^{***}$

Table S4. Uptake kinetic parameters of nitrogen uptake by *Zostera marina* plant parts at three different temperatures (8, 12 and 17 ºC). V_{max} = maximum uptake rate (µmol N g^{-1} dry weight h^{-1}); K_m = half-saturation constant (µM); and α = affinity constant (V_{max}/K_m). The coefficient of determination (r^2), level of significance (P) and the standard error of the estimate (values in brackets) are given. Data not displaying saturation kinetics were fitted with a linear regression model (V = uptake rate; S = substrate concentration).

	V_{max}	K_m	α	r^2	P
Ammonium (leaves)					
8 ºC	40.48 (16.53)	90.20 (64.87)	0.45	0.96	0.021
12 ºC	54.63 (5.18)	84.24 (14.42)	0.65	0.99	0.001
17 ºC	130.76 (25.89)	294.41 (74.16)	0.44	0.99	0.001
Nitrate (leaves)					
8 ºC	0.48 (0.08)	9.96 (7.12)	0.05	0.79	0.113
12 ºC	0.72 (0.06)	1.75 (1.27)	0.41	0.60	0.224
17 ºC	0.85 (0.11)	5.77 (3.77)	0.15	0.80	0.105
Alanine (leaves)					
8 ºC	3.20 (0.81)	76.95 (36.09)	0.04	0.98	0.011
12 ºC	4.19 (1.50)	118.18 (67.95)	0.04	0.97	0.014
17 ºC	3.43 (0.59)	48.69 (18.49)	0.07	0.98	0.012
Trialanine (leaves)					
8 ºC	$V = 0.063+0.005\ S$	-	-	0.99	0.073
12 ºC	$V = 0.108+0.007\ S$	-	-	1.00	0.012
17 ºC	0.72 (0.00)	11.44 (0.03)	0.06	1.00	0.001
	V_{max}	K_m	α	r^2	P
Ammonium (roots)					
8 ºC	9.66 (0.81)	40.38 (8.15)	0.24	0.99	0.004
12 ºC	19.39 (14.37)	111.61 (135.66)	0.17	0.87	0.069
17 ºC	$V = 1.113+0.119\ S$	-	-	0.99	0.004
Nitrate (roots)					
8 ºC	0.68 (0.13)	18.20 (11.77)	0.04	0.89	0.055
12 ºC	1.81 (0.30)	27.00 (2.70)	0.07	0.95	0.027
17 ºC	1.06 (0.14)	6.11 (4.20)	0.17	0.79	0.110
Alanine (roots)					
8 ºC	1.30 (0.34)	29.78 (21.59)	0.04	0.87	0.066
12 ºC	$V = 0.493+0.009\ S$	-	-	0.99	0.004
17 ºC	2.09 (0.22)	18.26 (6.32)	0.12	0.97	0.017
Trialanine (roots)					
8 ºC	$V = 0.257+0.011\ S$	-	-	1.00	0.010
12 ºC	0.88 (0.03)	2.96 (0.56)	0.3	0.98	0.089
17 ºC	1.57 (0.10)	5.82 (1.42)	0.27	0.98	0.090

Table S5. Results of the two-way ANOVA examining the effects of temperature (T) and nutrient concentration (N) on the nitrogen uptake rates of *Zostera marina*. Test statistic (F) and levels of significance are indicated as (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, (^{ns}) not significant.

	Temperature (T)	**Nutrient concentration (N)**	**T x N**
Leaves			
Ammonium	$F = 10.36^{***}$	$F = 124.00^{***}$	$F = 2.82^{*}$
Nitrate	$F = 9.26^{**}$	$F = 3.21^{*}$	$F = 0.76^{ns}$
Alanine	$F = 4.25^{*}$	$F = 37.84^{***}$	$F = 0.55^{ns}$
Trialanine	$F = 38.70^{***}$	$F = 61.99^{***}$	$F = 2.44^{ns}$
Roots			
Ammonium	$F = 12.30^{***}$	$F = 45.36^{***}$	$F = 12.23^{***}$
Nitrate	$F = 6.27^{**}$	$F = 4.37^{*}$	$F = 0.96^{ns}$
Alanine	$F = 20.03^{***}$	$F = 28.42^{***}$	$F = 1.40^{ns}$
Trialanine	$F = 26.91^{***}$	$F = 18.84^{***}$	$F = 1.80^{ns}$

Table S6. Whole-plant nitrogen budget (µmol m^{-2} h^{-1}) of *Zostera marina* along the species latitudinal distribution range in Europe, calculated using the nitrogen uptake rates (µmol g^{-1} dry weight h^{-1}), areal biomass (g^{-1} dry weight m^{-2}) and ambient nutrient concentrations (µM) determined at each site (Iceland, United Kingdom and Portugal). Values in brackets show the percentage contribution of each nitrogen source to the plant's total N acquisition. DIN = dissolved inorganic nitrogen, DON = dissolved organic nitrogen.

	Iceland	United Kingdom	Portugal
Leaves			
Ammonium	4.6 (2.1 %)	91.6 (20.4 %)	94.8 (13 %)
Nitrate	4.5 (2.0 %)	9.9 (2.2 %)	91.8 (12.6 %)
Amino acids	0.8 (0.3 %)	4.4 (1.0 %)	3.2 (0.4 %)
Peptides	4.7 (2.1 %)	21.3 (4.8 %)	31.3 (4.3 %)
DIN	9.1 (4.1 %)	101.4 (22.6 %)	186.6 (25.6 %)
DON	5.4 (2.4 %)	25.7 (5.7 %)	34.5 (4.7 %)
Roots			
Ammonium	146.6 (65.8 %)	197.7 (44.1 %)	300.8 (41.2 %)
Nitrate	3.9 (1.8 %)	2.4 (0.5 %)	35.2 (4.8 %)
Amino acids	25.9 (11.6 %)	70.6 (15.7 %)	57.6 (7.9 %)
Peptides	31.8 (14.3%)	50.5 (11.3 %)	115.3 (15.8 %)
DIN	150.5 (67.6 %)	200.1 (44.6 %)	336.1 (46.0 %)
DON	57.7 (25.9 %)	121.1 (27.1 %)	173.0 (23.7 %)
Whole-plant			
Total DIN	159.6 (71.7 %)	301.5 (67.2 %)	522.7 (71.8 %)
Total DON	63.1 (28.3 %)	146.8 (32.8 %)	207.5 (28.2 %)

971 Table S7. Whole-plant nitrogen budget (µmol m^{-2} h^{-1}) of *Zostera marina* at three
972 different temperatures (8, 12 and 17 ºC), calculated using the nitrogen uptake rates
973 (µmol g^{-1} dry weight h^{-1}) obtained at each temperature, areal biomass (g^{-1} dry weight m^{-2})
974 and ambient nutrient concentrations (µM) at the intermediate site (United Kingdom).
975 Values in brackets show the percentage contribution of each nitrogen source to the
976 plant's total N acquisition. DIN = dissolved inorganic nitrogen, DON = dissolved
977 organic nitrogen.
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	8 ºC	12 ºC	17 ºC
Leaves			
Ammonium	63.4 (16.4 %)	91.6 (20.4 %)	63.1 (12.3 %)
Nitrate	1.2 (0.3 %)	9.9 (2.2 %)	3.7 (0.7 %)
Amino acids	5.1 (1.3 %)	4.4 (1.0 %)	8.6 (1.7 %)
Peptides	12.6 (3.3 %)	21.3 (4.8 %)	11.0 (2.1 %)
DIN	64.6 (16.7 %)	101.4 (22.6 %)	66.8 (13.0 %)
DON	17.7 (4.6 %)	25.7 (5.7 %)	19.6 (3.8 %)
Roots			
Ammonium	214.6 (55.4 %)	197.7 (44.1 %)	243.3 (45.6 %)
Nitrate	1.3 (0.3 %)	2.4 (0.5 %)	5.7 (1.1 %)
Amino acids	58.9 (15.2 %)	70.6 (15.7 %)	108.3 (21.1 %)
Peptides	30.3 (7.8 %)	50.5 (11.3 %)	79.2 (15.4 %)
DIN	215.9 (55.7 %)	200.1 (44.6 %)	240.0 (46.7 %)
DON	89.2 (23.0 %)	121.1 (27.0 %)	187.4 (36.5 %)
Whole-plant			
Total DIN	280.5 (72.4 %)	301.5 (67.2 %)	306.8 (59.7 %)
Total DON	106.9 (27.6 %)	146.8 (32.8 %)	207.0 (40.3 %)

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992 Figure S1. Aspect of the *Zostera marina* meadows in (a) Iceland (high-latitude), (b)
993 United Kingdom (mid-latitude), and (c) Portugal (lower-latitude) during summer at low
994 tide.
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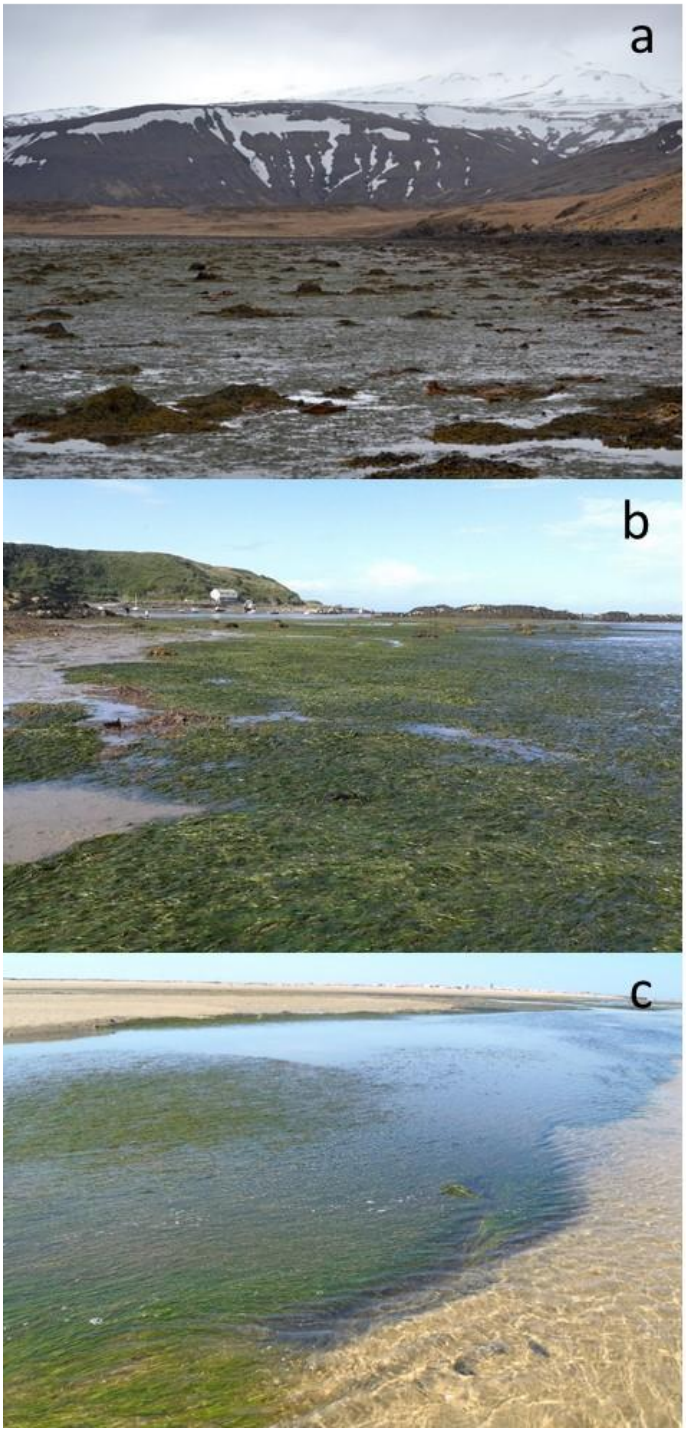

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